**Assay for Beryllium Hypersensitivity**
Chronic Beryllium Disease (CBD) affects the lungs. It is caused by an abnormal immune response to inhaled beryllium in 1 to 5% of beryllium workers. A hallmark of CBD is immune hypersensitivity to beryllium. Hypersensitivity to beryllium and other antigens can also be measured in the laboratory using blood samples. The authors have developed an improved blood lymphocyte proliferation test, based on flow cytometry for measuring an individual's hypersensitivity to beryllium. Human blood-derived cell cultures can possibly contain blood-borne pathogen(s) that cause infection.

**Detection and Identification of Bacteria**
This project is developing methods for detection and identification of bacterial species that are related to potential biological threat agents, as well as for forensic applications. Our detection strategy uses flow-cytometry of DNA fragments generated either directly by enzyme digestion of bacterial DNA or by PCR amplification of specific bacterial sequences. Closely related species to select agent pathogens, as well as common food pathogens, can sometimes cause infection. Select agent pathogens will not be studied.

**Genetic Studies of Subgroup 1 Bacilli**
Genetic studies of *B. anthracis*, the causative agent in anthrax, and closely related bacterial species require production of sufficient DNA to conduct the molecular genetic analyses that characterize these microbes. DNA from different *B. anthracis* isolates is produced by an outside collaborating laboratory, then sterilized by filtration certified free of bacteria or spores and then shipped to LANL. *B. cereus* and *B. thuringiensis* isolates are handled at LANL. These species and strains can sometimes cause infection.

**Producing Proteins Using Vaccinia virus**
In this proposal, we are using the vaccinia virus gene expression system to make large quantities of non-vaccinia proteins for biochemical study. These non-hazardous proteins will be purified from infected human cultured cells to measure enzyme activities and binding to other proteins. Vaccinia virus itself can sometimes cause infection.

**Culturing *Yersinia enterocolitica* and *Yersinia pseudotuberculosis***
*Yersinia enterocolitica* and *Y. pseudotuberculosis* are closely related to *Y. pestis*, the causative agent of plague. However, unlike their relative, these two microbes cause illnesses that are self-limiting and only rarely severe. Our phylogenetic studies of *Y. pestis* require that we compare it genetically to other closely related microbes in the environment. These comparisons are done at the DNA level and they do not require live microbes. Certified sterile, *Y. pestis* DNA is provided to us by a collaborating outside laboratory. *Y. enterocolitica* and *Y. pseudotuberculosis* can sometimes cause infection.

**Genetic and Physiologic Properties of *Bacillus* species**
An important part of understanding biothreat agents is the genetic and physiologic properties of closely related species. Several *Bacillus* species have been identified that share genetic features with *B. anthracis* but do not cause anthrax. Our lab studies the DNA sequence, gene expression, of these Bacilli. The Bacillus species can sometimes cause infection.

**Comparison of Methods for Bacterial Strain Identification**
Under a project called the Bioforensics Demonstration and Application Program (BDAP) we are conducting an evaluation of several technology platforms for pathogen strain identification. A major focus of the project is the generation of a database of DNA fingerprints for several known pathogens and subsequent matching of unknowns selected from the original strain panels to the database. Some bacterial species studied here can sometimes cause infection.

**Host-Pathogen Interactions with *Yersinia enterocolitica***
In this proposal, we are studying how the food-borne bacteria *Yersinia enterocolitica* stimulates human host cells. Study of how human cells respond to the bacteria may give us insights into prevention and treatment of food poisoning outbreaks. Y. enterocolitica can sometimes cause infection.

**Immune Cell Biosensor for Rapid Pathogen Detection and Identification**
We are developing an assay for the detection and identification of pathogens based on specific interactions with immune cells in culture. Current efforts are focused on proof-of-principle using human monocyte cell lines engineered to contain a reporter construct which is activated upon binding a pathogen. Initial work will use 'model' pathogens including *E. coli*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus*. These organisms can sometimes cause infection.

**The Impact of Different Bacteriophage on *B. anthracis***
Bacteriophage are viruses that only infect bacteria. Like their microbial hosts, bacteriophage are genetically very diverse but each type has a limited host cell range. Bacteriophage that can, for example, infect and kill *E. coli*, usually cannot infect and kill other species of bacteria. Our studies focus on the identification of bacteriophage that can infect and kill *B. anthracis*. BSL-2 containment is appropriate 1) to minimize cross-contamination of laboratories with phage virus. 2. *B. anthracis* (Sterne) is a laboratory strain which is non-pathogenic, but other closely related Bacillus species can sometimes cause infection.

**Immunoassay for Rapid Influenza Virus Typing**
We are developing microsphere-based flow cytometric assays to be used for the rapid typing and subtyping of influenza virus strains and substrains. Successful development of these assays would improve diagnosis and treatment of influenza infections and also assist in following the spread of an infection through a population. The influenza strains used here can sometimes cause infection.

**Early Diagnosis of Infection**
The work aims at developing fluorescence-based assays for the detection of influenza viruses by fluorimetry and flow cytometry, and integration of these assays into compact and easy to use sensing devices. The assay development will involve the preparation of fluorescently labeled receptor molecules that recognize human influenza, and testing of such receptors for binding of both inactivated human influenza viruses and live human viruses obtained from tissue culture and clinical specimens. Tissue samples could be contaminated with human pathogens. The flu virus strains can sometimes cause infection.

**Optical Diagnosis of Cancer**
The scientific goal of this project is to develop non-invasive, optical methods for cancer diagnosis and treatment monitoring. Optical measurement involves exposing the cells to a laser or broad-band light source, then measuring various properties of the scattered and/or absorbed light. In order to characterize optical signals specific for malignancy, we need to compare normal epithelial cell lines to cell lines either derived from human tumors or those that have undergone malignant transformation through transfection with oncoproteins. Human tumor cell lines may conceivably contain pathogens capable of laboratory infection.

**Human Susceptibility to Chronic Beryllium Disease**
In this proposal, we will be obtaining immune cell samples of patients with Chronic Beryllium Disease to study the cellular differences between healthy and diseased individuals. This work will allow us to determine why some individuals are more susceptible to this disease compared to the general population. Human primary immune cells may sometimes contain pathogens capable of laboratory infection.

**Culture and Processing of *B. anthracis* and *Y. pestis***
Pathogen surrogates have value as a substitute for infectious organisms and have been used in B division research for several years. The goal of this capability is to culture and process the microbiological pathogens, *B. anthracis* and *Y. pestis*, in Bioscience Division's BSL-2 Select Agent Laboratories as described in the CDC guidelines. This capability will enhance LANL's ability to respond to its threat reduction mission. This work will be subject to Biological Safety Officer coordination of on the job training (OJT) to ensure safe handling of these select agent pathogens. These pathogens can sometimes cause infection.

**Separation of Tumor Cells from Whole Blood**
Microfluidic magnetic separation channels are to be tested for their ability to separate model tumor cells from diluted whole animal blood. Cultured HT-29 colon carcinoma cells will be both fluorescently and magnetically tagged using specific antibody probes. The cells will be spiked into citrated sheep or rabbit blood samples and pumped through the microfluidic devices which

will be analyzed using a fluorescent microscope. The human cell cultures may contain pathogens capable of laboratory infection. The ovine or bovine blood samples must be tested free of specific human pathogens.

**Genetic Mechanisms for Reduction of Virulence in Bacillus anthracis**

The broad goal of this project is to develop simple, effective strategies to generate alternative vaccine candidate strains from cultures of pathogenic B. anthracis bacterium. Using Bacillus anthracis Sterne (the attenuated live vaccine strain) as a model system, the project applies both classical and innovative procedures to identify derivative strains that are thoroughly non-pathogenic less than the parent vaccine strain. B. anthracis (Sterne) is itself non pathogenic, but occasional genetic variants produced could conceivably sometimes cause infection.

**Generation of Antibody Libraries from SARS-Infected Individuals**

This proposal outlines the use of human blood samples from normal individuals or those infected (and convalescent) with SARS virus for the generation of libraries of antibody genes. Libraries of such antibody genes can be used to isolate antibodies recognizing targets of interest. Derived antibodies have the potential to be used for therapeutics, and may also eliminate the need for animal immunization to obtain antibodies of interest.

**Human Cell Response to Foreign Stimuli**

In this proposal, we are studying the response of human cells to various foreign stimuli, such as *Staphylococcal aureus* superantigens, which cause food poisoning, and beryllium, which causes Chronic Beryllium Disease. Study of the molecular mechanisms by which these antigens stimulate the disease state will give us an understanding to combat these diseases. Human cell materials and S, aureus can sometimes cause infection.